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Immune responses of poultry to Newcastle disease virus ☆

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ABSTRACT

Newcastle disease (ND) remains a constant threat to poultry producers worldwide, in spite of the availability and global employment of ND vaccinations since the 1950s. Strains of Newcastle disease virus (NDV) belong to the order Mononegavirales, family Paramyxoviridae, and genus Avulavirus, are contained in one serotype and are also known as avian paramyxovirus serotype-1 (APMV-1). They are pleomorphic in shape and are single-stranded, non-segmented, negative sense RNA viruses. The virus has been reported to infect most orders of birds and thus has a wide host range. Isolates are characterized by virulence in chickens and the presence of basic amino acids at the fusion protein cleavage site. Low virulent NDV typically produce subclinical disease with some morbidity, whereas virulent isolates can result in rapid, high mortality of birds. Virulent NDV are listed pathogens that require immediate notification to the Office of International Epizootics and outbreaks typically result in trade embargos. Protection against NDV is through the use of vaccines generated with low virulent NDV strains. Immunity is derived from neutralizing antibodies formed against the viral hemagglutinin and fusion glycoproteins, which are responsible for attachment and spread of the virus. However, new techniques and technologies have also allowed for more in depth analysis of the innate and cell-mediated immunity of poultry to NDV. Gene profiling experiments have led to the discovery of novel host genes modulated immediately after infection. Differences in virus virulence alter host gene response patterns have been demonstrated. Furthermore, the timing and contributions of cell-mediated immune responses appear to decrease disease and transmission potential. In view of recent reports of vaccine failure from many countries on the ability of classical NDV vaccines to stop spread of disease, renewed interest in a more complete understanding of the global immune response of poultry to NDV will be critical to developing new control strategies and intervention programs for the future.

1. Introduction

Despite the advances made in the diagnosis of and vaccination for Newcastle disease since it was first described in 1926, the disease continues to negatively impact poultry producers by infecting birds worldwide (Alexander et al., 2012; Goldhaft, 1980). From 2006 to 2009 the most widespread animal diseases, in terms of the number of countries affected, were rabies, Newcastle disease (ND) and Bovine tuberculosis (Anonymous, 2011). ND ranked as the fourth most important disease in terms of the number of livestock units lost for poultry species, behind highly pathogenic avian influenza, infectious bronchitis, and lowly pathogenic avian influenza (Anonymous, 2011). The disease is caused by only the virulent strains of avian paramyxovirus serotype-1 (AMPV-1) and APMV-1 is synonymous with Newcastle disease virus (NDV) (OIE, 2012). Strains are defined as virulent if they (1) have three or more basic amino acids at position 113–116 of the un-cleaved fusion protein cleavage site (F0) with a phenylalanine at position 117, or (2) obtain a intracerebral pathogenicity index (ICPI) value of $\geqslant$0.7 in day-old chickens (*Gallus gallus*) (OIE, 2012). Failure to demonstrate multiple basic amino acids necessitates an ICPI value be obtained for the isolate.

NDV is known to infect over 236 species of birds (Kaleta and Baldauf, 1988) and besides poultry species virulent NDV (vNDV) strains are commonly found in pigeons and double crested cormorants (Diel et al., 2012b; Kim et al., 2008; Pchelkina et al., 2013) and occasionally in some other wild bird species (Kaleta and Kummerfeld, 2012). Typically, the concern is that pigeons will transmit their vNDV strains of genotype VIb to poultry (Abolnik et al., 2004; Alexander and Parsons, 1986), however, poultry are able to transmit their vNDV strains to pigeons, as well (Merino et al., 2009). The incubation period and clinical disease observed with a NDV infection depends on multiple factors. The typical

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range is from three to six days depending on the species of host infected with the vNDV, the immunity of the host to NDV and the amount and strain of vNDV the host is exposed to (Alexander and Senne, 2008).

The clinical signs observed upon infection will be non-specific and can include depression, ruffled feathers, open mouth breathing, hyperthermia, anorexia, listlessness and hypothermia before death. In addition, since the lesions observed upon infection with vNDV are not pathognomonic, other diseases such as highly pathogenic avian influenza, infectious laryngotracheitis and mycoplasmosis should be considered (Alexander and Senne, 2008). However, if hemorrhage and necrosis of lymphoid tissues is present, especially of the intestine, spleen and thymus, viscerotropic vNDV should be suspected (Cattoli et al., 2011). Because layers receive multiple NDV vaccinations during their production cycle, and thus have persistent immunity, they may not show signs of infection except a drop in egg production (Bwala et al., 2012; Cho et al., 2008). Birds infected with neurotropic vNDV strains remain alert prior to developing neurological signs such as torticollis, ataxia or a wing or leg paralysis and gross lesions are usually absent (Cattoli et al., 2011).

All strains of Newcastle disease virus (NDV) belong to the order Mononegavirales, family Paramyxoviridae, and genus Avulavirus, are contained in one serotype and are also known as avian paramyxovirus serotype-1 (APMV-1) (Alexander and Senne, 2008). The virions are pleomorphic in shape, and consist of single-stranded, non-segmented, negative sense RNA genomes (Miller et al., 2010). There are at least three different genome lengths (15,186, 15,192 or 15,198), with six genes that produce six structural proteins in a 3′ to 5′ order: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN) and the RNA dependent RNA (large) polymerase (L). Editing of P produces at least one other protein, the V protein, which has anti-interferon properties (Czegledi et al., 2006).

Even though all strains of NDV are contained in one serotype, there are phylogenetic differences found when comparing genome relatedness. Strains are divided into two classes, class I and class II, with class II further divided into 16 genotypes (Diel et al., 2012a). Class I viruses are typically isolated from wild birds and all reported strains are of low virulence except for one strain, chicken/Ireland/1990 (Alexander et al., 1992). Class II, genotype I NDV are all of low virulence except for the vNDV that caused the ND outbreak in 1998 in Australia (Gould et al., 2001). Class II, genotype II viruses contain NDV of low virulence, some of which (B1, LaSota, VG/GA) are used as NDV vaccines, and vNDV that are not commonly isolated (Miller et al., 2010). NDV strains of class II, genotypes III–IX, and XI–XVI are all virulent (Courtney et al., 2012; Diel et al., 2012a). Isolates of class II, genotype X are of low virulence and most often found in wild birds, but some have been isolated from some poultry species (Diel et al., 2012a; Miller et al., 2011).

While humoral immunity from vaccination is critical to ND control, another important aspect that is not a new concept, but is often neglected, is the differences in resistance to ND due to genetic variation (Albiston and Gorrie, 1942). In addition, it is known that there is a negative correlation between a primary antibody response to NDV and favorable production traits (Lwelamira et al., 2009). Genetic resistance to ND has been observed with various lines within a breed for chickens (Cole and Hutt, 1961; Gordon et al., 1971) and turkey (Tsai et al., 1992) and among breeds of chickens (Hassan et al., 2004; King, 1996) and ducks (Shi et al., 2011). Concerning this topic it is important to note that each Newcastle disease virus may be better adapted to grow in one species versus another, like what is seen with PPMV1 (pigeon NDV) strains in chickens (Pearson et al., 1987). Another example of this can be seen with the variability in the bird infectious dose 50 of one NDV for chickens, turkeys and ducks (Aldous et al., 2010). While improving genetic resistance to ND through breeding more resistant bird strains appears to be feasible, logistically it is very difficult due to the involvement of multifactorial components. Perhaps when the efficiency of producing transgenic birds is improved, more disease resistance breeds can be used for this purpose (Zhang et al., 2012).

Another important factor for ND control in developing countries is the lack of a "cold chain" or reliable source to keep the vaccines at 4 °C. Even the best live vaccine will not induce an immune response if it is not viable due to improper storage during the distribution process. Progress has been made with the thermostable I-2 strain of NDV and has been put into place in some developing countries (Bensink and Spradbrow, 1999; Harrison and Alders, 2010; Illango et al., 2005; Nasser et al., 2000). Continued improvement and utilization of thermostable NDV strains is necessary to improve controls in countries where vNDV isolates are endemic and the cold chain is unreliable.

2. Innate immune response to NDV infection in poultry

The innate immune response comprises factors that exist prior to the advent of infection, and are capable of exclusion or rapid response to microbes. The primary components of innate immunity of poultry are (1) physical and chemical barriers, such as feathers and skin, epithelia and production of mucus; (2) phagocytic cells, including macrophages and natural killer cells; (3) complement proteins and mediators of inflammation; and (4) cytokines. Overall, the innate immune response to virus infection is an immediate reaction designed to control and inhibit virus growth and spread and aid in developing pathogen-specific protection through the adaptive immune response. The early reactions of the innate immune system use germ-line encoded receptors, known as pattern recognition receptors (PRR's), which recognize evolutionarily conserved molecular markers of infectious microbes, known as PAMP's (pathogen-associated molecular patterns). Recognition of PAMPs by PRRs, either alone or in heterodimerization with other PRRs, (toll-like receptors (TLR); nucleotide-binding oligomerization domain proteins (NOD); RNA helicases, such as retinoic acid-inducible gene 1 (RIG-I) or MDA5; C-type lectins), induces intracellular signals responsible for the activation of genes that encode for pro-inflammatory cytokines, anti-apoptotic factors, and antimicrobial peptides. The virus is first recognized by host sentinel proteins, including TLR and NOD proteins, producing rapid signaling and transcription factor activation that lead to production of soluble factors, including interferon and cytokines, designed to limit and contain viral replication.

NDV infection *in vitro* results in nitric oxide (NO) induction in chicken heterophils and peripheral blood mononuclear cells, interferon alpha (IFN-α) and beta (IFN-β) mRNA detection in macrophages, and gamma (IFN-γ) mRNA production in peripheral blood mononuclear cells (Ahmed et al., 2007; Sick et al., 2000, 1998). In addition, infection of chicken heterophils decreased the ability to phagocytose bacteria, resulting in impaired heterophil function, and making birds more susceptible to secondary infection (Lam et al., 1996). Constitutive low-level expression of NO in the vascular endothelium plays a beneficial role in maintaining blood vessel homeostasis, but high levels of NO produced by macrophages in response to pathogens can have toxic effects on the host (Palmer et al., 1987).

In mammalian systems, such as cultured murine macrophages, NDV induced both IFN-α and IFN-β (Hoss et al., 1989; Zawatzky et al., 1991). The functional significance of the interferon regulatory factor genes (IRF)-3 and IRF-7 was examined in mouse macrophages derived from deletion knock out (KO) animals (Wilden

et al., 2011). Deletion of the IRF-3 or IRF-7 gene increased the susceptibility of mouse macrophages to NDV infection. NDV replicated better in IRF-3 KO than in IRF-7 KO macrophages. Furthermore, early production of type I interferon at later time points, as opposed to high maximal levels, appears important for resistance to NDV infection. Taken together, these results demonstrate a role for IRF-3 in the innate anti-viral response to NDV of mouse macrophages.

Using microarray systems to measure gene expression patterns in chicken embryo fibroblasts infected with vNDV, strain Texas GB, Munir, et al. demonstrated increased IFN-α and IFN-β (Munir et al., 2005). Stimulation of interferon resulted in the upregulation of numerous interferon-stimulated genes (ISG), include the IFN induced protein with tetratricopeptide repeats 4, retinoic acid and IFN inducible 58 kDa protein (RI58), IFN-induced 56 kDa protein (IFI-56K), IFN-α inducible protein P27-H, and signal transducer and activator of transcription-1 (STAT-1α/β). Interestingly, in these studies peak gene expression *in vitro* did not appear until 36 h post infection (h.p.i.), unlike other studies that show earlier innate expression.

Our *in vitro* studies confirm those of others, demonstrating the strong induction of the host response genes, IFN-α, IFN-β, interleukin (IL)-1β and IL-6 in splenic leukocytes (Rue et al., 2011). Using real-time RT-PCR of RNA isolated from NDV-infected splenocytes, we have demonstrated that the vNDV strain, CA02, but not the lentogenic LaSota virus, at 6 h.p.i. is capable of rapidly and strongly inducing IFN-α, IFN-γ, IL-6 and IL-1b, genes integral to a pro-inflammatory response (Fig. 1). Unfortunately, since LaSota does not reach the spleen but only replicates at the site of inoculation, parallel *in vivo* experiments to compare the relative host response to virulent and LaSota NDV strain of low virulence were not possible (Wakamatsu et al., 2006a,b). The observed differences in time of cytokine expression between our studies and others may be explained by differences in the dose of virus used between the studies. The enhanced host response to vNDV, in conjunction with severe pathological damage observed, is somewhat surprising considering that all NDV encode a gene, V, which functions to suppress class I IFNs. However, this robust innate response to vNDV has been suggested as being deleterious to the host, and a possible cause of the pathological effect (Rue et al., 2011). Regardless, it is clear that NDV of low virulence stimulates a lower innate immune responses compared with vNDV that induces significantly higher levels.

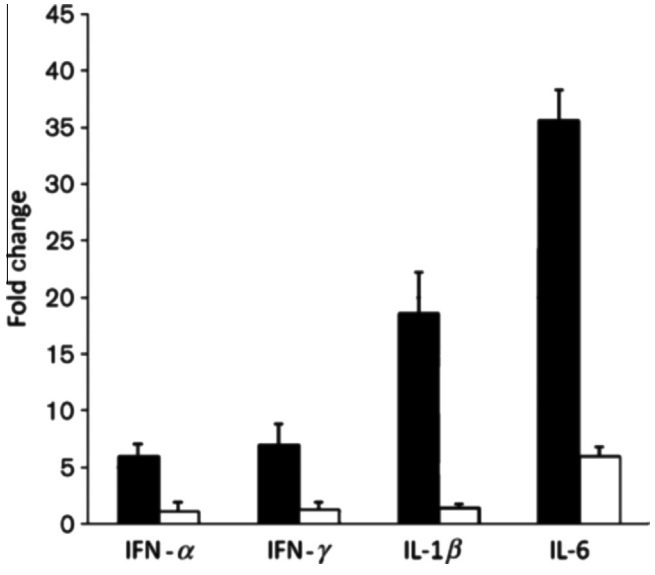

Fig. 1. Real-time RT-PCR of NDV-CA02 and LaSota vaccine strain-infected chicken splenocytes *in vitro*. Total RNA isolated from chicken splenocytes infected with NDV, CA02 and LaSota, for 6 h was used for real-time RT-PCR using SYBR Green for IFN-α, IFN-γ, IL-1β and IL-6. Results were normalized against 28S rRNA and fold-changes relative to mockinfected samples are shown. Open bar represents low virulent LaSota and solid bar represents vNDV CA02 (CA02).

While useful, the expanding knowledge of the *in vitro* response to NDV is insufficient to understand the nature of the host response to vNDV *in vivo* or to relate the underlying host immune mechanisms to viral pathogenesis. Despite decades of research characterizing pathogenesis of different isolates, little is known about the molecular mechanisms of disease caused by NDV in the host. Recently, global analysis of the host response to infection *in vivo* with a vNDV strain from the 2002 outbreak in California (CA02) characterized the early host response in chickens (Rue et al., 2011). Using microarray technology, a strong transcriptional host response in spleens of chickens at early times after infection was demonstrated with the induction of groups of genes involved in antiviral and pro-inflammatory cytokine responses. Multiple genes were upregulated at 48 h.p.i. including, IFN-α, IFN-γ, several cytokines and chemokines, IFN effectors and inducible nitric oxide synthase (iNOS). Many genes associated with an early innate host response were induced by CA02 at 24 h.p.i., including the pro-inflammatory cytokine IL-6, chemokine macrophage inflammatory protein-3 alpha (MIP-3a), myxovirus resistance gene (Mx), lysozyme, interferon-induced protein with tetratricopeptide repeats 5, ISG12-2, melanoma differentiation associated protein-5, and IFN-γ precursor. Several other markers of the innate immune response to NDV that were not induced at 24 h.p.i. were upregulated at 48 h.p.i., including iNOS, IL-1β, IL-18, IL-8 and IFN-γ. The increased transcription of iNOS was confirmed by immunohistochemistry in spleens and measured levels of nitric oxide in serum, indicating functional protein formation. The significant upregulation of iNOS in spleens and NO in serum is a potentially destructive innate response of chickens to NDV infection, but in light of the robust replication and rapid mortality of this virus in chickens, it seems quite possible that NO is contributing to mortality not recovery.

Finally, vNDV infection also induced expression of the antiviral IFN effector genes, namely protein kinase R and 2′-5′-oligoadenylate synthetase (OAS). Other cytokines, including K203, ah221, CXCL13/BCA-1, CCL21, and MIP-1β, were also upregulated following NDV infection. Several of these cytokines are chemokines, most notably MIP-3α and MIP-1β, which function to enhance cell-mediated responses by recruiting effector leukocytes. Other genes that were significantly induced by the CA02 infection are part of the innate signaling processes, including regulator of G-protein signaling 1 (ADORA), suppressor of cytokine signaling (SOCS)-1 and -3, N-myc, STAT interactor, STAT4 and IRF 1, 7 and 10.

3. Antibody response to infection and vaccination with NDV

In addition to biosecurity and the culling of infected birds, vaccinations are a critical component to control ND (Marangon and Busani, 2006; Seal et al., 2000). International and national vaccination control policies will depend on the factors affecting that sector of poultry production, while keeping with the OIE regulations (OIE, 2012). The goal of vaccination is always sterilizing immunity, however, that has not yet been achieved with NDV vaccines. At best, NDV vaccines induce an immune response that reduces or completely prevents clinical disease and mortality from ND, decreases the amount of vNDV shed into the environment, and increases the amount of virus needed to infect the vaccinated animal (Marangon and Busani, 2006; Miller et al., 2009).

Herd immunity is another beneficial consequence of a successful vaccination program as it provides some protection to

suboptimal-vaccinated or unvaccinated birds in an otherwise well-vaccinated flock (Marangon and Busani, 2006). However, this outcome is only achieved with ND when greater than 85% of the flock have hemagglutination inhibition (HI) antibody titers greater than 8 after two vaccinations (van Boven et al., 2008). Field results suggest that only birds with HI titers greater than 16 after multiple vaccinations will survive a vNDV challenge as 66% of the flock succumbed with titers less than that (Kapczynski and King, 2005). More commonly, HI levels of 32 or higher are what are typically thought of being protective (Allan et al., 1978).

Mass application of live vaccines is often used due to the lower cost and faster application time compared to having to administer individual vaccines to each bird of a flock (Senne et al., 2004). The lentogenic B1 and LaSota vaccine strains of low virulence are commonly used worldwide, and can provide protection against vNDV if the vaccines are viable, administered correctly to healthy birds and time is allowed for an appropriate immune response to develop prior to exposure to the challenge virus (Cornax et al., 2012; Dortmans et al., 2012; Kapczynski and King, 2005). Unfortunately, conditions in the field are often less than optimal with mass application potentially reaching as little as 53% of the flock when the route of administration is spray and 60% when the route is through the drinking water (Degefa et al., 2004). The presence of immunosuppressive organisms can also render ineffective a protocol that is sound under experimental conditions (Perozo et al., 2012). Indeed even the most efficacious vaccine cannot induce an immune response if the bird is immunosuppressed.

Inactivated vaccines are often administered to layers and breeders to provide long lasting high antibody titers that can be passed to offspring (Al-Garib et al., 2003b). However, withdrawal times between vaccination and slaughter reduce the ability to use these types of vaccines throughout the production period (Senne et al., 2004). Inactivated vaccines are more expensive to produce, and require individual administration. Until recently the dogma is that inactivated vaccines will not induce a mucosal immune response, but a recent study demonstrated that both live and inactivated NDV vaccines induced antibodies other than IgA, not only in serum, but also in tracheal and intestinal washes (Chimeno Zoth et al., 2008).

Because all NDV are in one serotype any NDV strain can be used as a vaccine and all vaccines should prevent clinical disease and death from ND. However, some studies have been demonstrated that vaccines formulated with strains more similar to the challenge virus can decrease the amount of challenge virus shed in oropharyngeal swabs from vaccinated birds and potentially decrease the number of birds that shed virus (Cho et al., 2008; Miller et al., 2009, 2007; Xiao et al., 2012). These experiments demonstrate that some NDV strains are more antigenic than others from varying HI antibody titer levels to different antigens after equal amounts of vaccines are administered. Testing the same hypothesis, using additional experimental recombinant NDV strains, others have found no difference in the shedding of vNDV in tracheal swabs after challenge and maintain that the inadequate application of NDV vaccines worldwide account for the current outbreaks and spreading of vNDV field strains (Dortmans et al., 2012). Due to all NDV being one serotype, a vaccine formulated with a new strain would not perform worse than a traditional strain and might possibly decrease the amount of vNDV shed into the environment from the vaccinated birds. Considering that mass vaccine applications methods are necessary for commercial poultry, support for newer NDV vaccines to provide better protection is needed.

In ovo vaccination at the time when eggs are moved from the incubators to the hatcher is an attractive option for poultry producers. Marek's disease vaccines, infectious bursal disease vaccines and fowl pox vaccines are commonly delivered at this time (day 18 or 19 of incubation) using egg injection systems (Williams and Zedek, 2010). Unfortunately little progress has been made with attenuating live NDV strains to give consistently reliable hatchability results (Dilaveris et al., 2007; Kapczynski et al., 2012; Ramp et al., 2012). However, the recombinant HVT (rHVT) platforms containing the fusion (F) gene of NDV is promising for *in ovo* use (Anonymous, 2010; Palya et al., 2012). For areas with endemic vNDV other traditional vaccines may have to be delivered along with the rHVT (Rauw et al., 2010). Unfortunately, while some HI activity may be present the ELISA and HI assays cannot be used to assess the immunity induced by these rHVT vaccines and efficacy is determined with mortality data. *In ovo* administration of fowl pox (FP) vectored ND vaccines is also possible (Sharma et al., 2002). These rHVT and FP vectored vaccines are advantageous in that they do not cause respiratory disease after vaccination as live NDV vaccines may. Racing pigeons are often vaccinated with killed ND vaccines formulated with inactivated a PPMV-1 strain, which decreases the duration, incidence and amount of virus shed after challenge, which is one of the goals of vaccinating for any disease (Kapczynski et al., 2006). Even though vaccinated birds infected with vNDV will shed virus after infection, with vNDV first shed in oral secretions peaking three to four days post infection and cloacal shedding occurring later (Miller et al., 2009), the goal of vaccination should be to decrease shedding two to three logs compared to non-vaccinated animals (Miller et al., 2007).

In the chicken, IgM, IgY (avian IgG equivalent) and IgA antibodies are produced as part of the immune response (Jeurissen et al., 2000). Antibodies are detected at the site of infection and in the blood starting at six days after infection or live virus vaccination and peaks 21–28 days after infection (Al-Garib et al., 2003a). Antibodies neutralize the ND virus particles by binding and preventing attachment of the virus to host cells (Al-Garib et al., 2003a). Approximately 30% of the IgY and 1% of the IgM and IgA antibodies present in the hen's plasma will passively transfer to the offspring and if the NDV antibody levels are high enough can provide protection until the levels fall below a protective level (Hamal et al., 2006). This maternal antibody can interfere with live vaccination by neutralizing the vaccine virus (Giambrone and Closser, 1990; Westbury et al., 1984).

Adjuvants to improve the immune response of NDV vaccines were initially focused on inactivated vaccines (Mitchell and Walker, 1951; Stone and Xie, 1990; Yin et al., 2006), but now include substances to favorably modulate the immune response from live NDV vaccines (Hilton et al., 2002; Zhang et al., 2007). Dietary supplements are commonly tested because the compounds may be locally available and/or because the compound maybe easily added to the diet to improve the immunity after vaccination. Lactobacillus-based probiotics have been shown to improve humoral immunity to live NDV vaccines in birds under heat stress (Sohail et al., 2010). Antibiotics may be added to water at the time of vaccination to provide an undefined benefit to the birds (Khalifeh et al., 2009). However, when antibiotics are evaluated for their ability to positively potentiate the humoral immune response to NDV vaccines, typically they are found to decrease the response (Khalifeh et al., 2009) or not significantly improve the response (Munir et al., 2007). Astragalus polysaccharides commonly used in Chinese medicines to enhance the immune response demonstrated slight improvements in the humoral immune response to NDV vaccination with or without sulfation (Huang et al., 2008). Glycyrrhetinic acid liposomes demonstrated a significantly improved humoral response to NDV vaccination 21–42 days after vaccination (Zhao et al., 2011).

4. Cellular immunity induced by NDV

Cell-mediated immunity (CMI) is specific adaptive immunity mediated by T lymphocytes and has been suggested to be an

important factor to the development of protection in chickens vaccinated against NDV and contribute to viral clearance (Cannon and Russell, 1986; Ghumman et al., 1976; Marino and Hanson, 1987; Merz et al., 1981; Perey et al., 1975; Sharma, 1999). The subsets of T lymphocytes, including cytokine-secreting CD4+ T helper cells, and CD8+ cytotoxic T lymphocytes (CTL), constitute the principal cells of the CMI response. Unlike antibody measurement via ELISA or HI, testing for CMI is more labor intensive and requires more skilled procedures. Tests for CMI include induction of IFN-γ from stimulated lymphocytes, cellular response to recall antigen or mitogen through proliferation, flow cytometry of lymphocytes, and levels of cytotoxicity observed by NDV specific CD8+ T cells to NDV-infected target cells. While the ability to measure avian CMI responses has steadily increased over the last few years, few studies have examined the induction and role of cell-mediated immunity in avian species against NDV.

Cell-mediated stimulation following NDV infection is detected as early as 2–3 days post infection (Ghumman et al., 1976). More recent studies also confirmed CMI responses to NDV may be detected shortly after vaccination with a live NDV vaccine (Reynolds and Maraqa, 2000). In those studies, chickens with CMI specific for NDV, determined by blastogenesis microassay with inactivated NDV, were not protected from lethal challenge in the absence of HI antibodies. However birds with NDV-specific antibodies were shown to be protected. The results indicate that antibodies are the key modulators of protection, but that CMI likely contributes to decrease viral shedding through target killing of NDV infected cells (Russell et al., 1997).

Subsequent studies have compared CMI responses between birds receiving live versus inactivated NDV vaccines. In one study, measurement of IFN-γ by ELISA and proliferation to NDV from splenocytes obtained from chickens receiving live or inactivated NDV vaccines were compared (Lambrecht et al., 2004). Results indicate increased CMI with the live NDV vaccination. Whereas live NDV stimulates both major histocompatibility complex (MHC) class I (CD8+) and II (CD4+) presentation in the host, CMI derived from inactivated NDV vaccines take longer to develop and are not as robust. CMI derived from inactivated vaccines appear to be stimulated through CD4+ lymphocytes and MHC-class II presentation that drive antibody formation likely through directed cytokine secretion. Additional studies examined the role of vaccine virulence in CMI. Not surprisingly, the virulence of the virus appears to play a role in CMI stimulation. Rauw et al., demonstrated an earlier and shorter CMI induced by a less virulent NDV vaccine strain, compared to a stronger and longer CMI mediated by a more virulent vaccines strain (Rauw et al., 2009). Thus, the more virulent strain persisted longer in the bird and therefore was able to increase magnitude and duration of CMI.

More recently, use of nanotechnology was employed to examine the adjuvant effect on inactivated NDV vaccines in chickens. In these studies, the addition of calcium phosphate (CP) was applied to inactivated NDV vaccines and the resulting CMI compared to that induced by live NDV vaccination (Koppad et al., 2011). Results indicate CMI induced with CP coupled to inactivated NDV achieved similar levels as those obtained with the live NDV vaccine. The results demonstrate that at 1-week post vaccination birds receiving CP significantly increased CMI to NDV compared to live NDV vaccinated birds. However, comparison of potency of the vaccines was not compared using direct challenge such that any positive contribution to protection through decreased viral shedding was not determined.

Taken together, recent advances in detection of CMI following live NDV vaccination make it apparent that while antibodies remain the primary mechanism of protection against virulent NDV, the contributions of CMI are important considerations in the face of field challenge. As new vaccine strategies are employed to protect poultry against vNDV it appears obvious that combining both arms of the adaptive immune response provide the best protection of birds and decrease the risk of transmission to susceptible animals.

5. Conclusion

NDV is an economically important and frequently isolated worldwide pathogen whose listed status with OIE marks its importance to both commercial poultry producers and poultry trading countries. Control of vNDV through use of vaccines is regularly and routinely practiced by all major poultry companies to provide immunological protection against disease. Our understanding of protective immunity against NDV is largely based on the production of antibodies directed viral proteins involved with attachment and fusion. However, our knowledge of the avian immune response to NDV is incomplete. While NDV exists as a single serotype, recent genotyping of vNDV isolates indicates that vaccines viruses established in the 1950s may be losing efficacy against these new viruses of the 21st century. There is a specific need for renewed research in immunity induced by NDV in poultry. One of the current challenges is to identify the molecular mechanisms of innate immunity that leads to an enhanced protection from infection and results in decreased shedding and transmission. Conversely, what are the deleterious effects of an unbalanced or unregulated innate immune response on pathogenesis, and how are viral factors contributing to this? Furthermore, the contributions of CMI to the overall protection against NDV, while undeniable, are still largely undefined. The cell types and epitopes involved need to be better characterized so that new vaccines can be designed to take advantage of this knowledge. With the advent of the genomics age, including the compete sequencing of the chicken genome, knowledge of structure and function of avian immune response elements involved in protective immunity to NDV can now be explored and tested. Elucidation of the immune response to NDV remains a top priority for the development of better control strategies in the face of reoccurring outbreaks.

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